Author's response to reviews

Title: Overexpression of NIMA-related kinase 2 is associated with progression and poor prognosis of prostate cancer

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Author's response to reviews: see over
Reviewer 1

1. **NEK2 may have a specific nuclear function connected to regulation of survival and apoptosis**
   We have added the discussion of the role of NEK2 in regulation of survival and apoptosis in the Introduction and Discussion as suggested.

2. **Need to discuss possible limitations of using one cell lines for the study in the results and conclusions.**
   The reason we chose LNCaP cells for the study is that LNCaP cell express NEK2 higher than other PCa cell lines. We fully agree with the reviewer that results derived from one cell lines suffer certain limitation. We have addressed this limitation in both Results and Discussion sections as suggested by the reviewer.

3. **How many independent experiments were done needs to be specified.**
   We have repeated the experiment 3 times and 5 mice per group were used. The description is added to the Method section.

4. **Why associations between NEK2 up-regulation and Gleason/stage are marginal in the Taylor dataset (RNA) but more evident in the IHC data need to be discussed.**
   The in silico analyses of the Taylor dataset are based on the expression at the RNA level and the IHC analyses are at the protein level. Transcription of the mRNA is not the only way to regulate gene expression. Both translational control and post translational control, as well as stability of the protein contributes to gene expression at the protein level. Therefore, it is not surprising that the different in RNA level was not as significant as in the protein level. We have added this in the Discussion section.

5. **The conclusion that NEK2 is of prognostic value for predicting outcome of PCa recurrence is an overstatement. Limitations of RNA-based screening and advantage of IHC-based screening need to be discussed.**
   The statements are revised and discussions are added as suggested.

6. **Need to describe that NEK2 has three splice variants (A-C) with possibly different roles in the Introduction.**
   Revised and one reference was added as suggested.

7. **What the definition of PSA recurrence needs to be described.**
   The definition is added the Method section.

8. **Details on the cell lines need to be included in the Materials/Methods section.**
   The detail information of the cell lines are added as suggested.

9. **Were normal prostate epithelial cells microdissected before RNA analysis? Was here NEK2 staining present in non-cancereous prostate cells, which might have an impact on**
RNA analysis?
We only used normal prostate tissue and PCa cell lines for the experiments. Yes, the presence of non-cancerous tissue may affect the RNA analyses. The issue is addressed in the discussion section.

10. It would be helpful to give the fraction of entirely negative (intensity 0) cancers and also of strongly positive cancers (i.e., 3+ in #50%).

Revised as suggested.

Reviewer 2

1. Overstatements in the introduction and discussion sections need to be toned down.
Revised as the suggestion.

2. How PSA recurrence, overall survival, death other than unexpected are defined needs to be described.
The overall survival was determined from the date of surgery to the time of the last follow-up or death. PSA recurrence was defined as three successive PSA rises (final value >0.2 ng/ml), single PSA >0.4 ng/ml, or use of secondary therapy administered for detectable PSA >0.1 ng/ml. The definitions are now added in the Methods section.

3. For the IHC analyses, why wasn’t percentage of positive cells considered on a continuous basis when generating the IRS score? There is no need to categorize the percentage of positive cells.
IRS score could be alternative to represent the IHC staining.

4. Fig 1a, Fig 1d, Fig 2c need p values.
P values are added to the figure legend.

5. The discussion “NEK2 knockdown results in compromised angiogenesis” has not data support.
Revised.

6. Mean IRS score for NEK2Fig 2d-e needs to be added. P values are needed and remove the statement ‘NEK2 expression is obviously downregulated’.
IRS score was added as suggestion. P values are added and the statement is removed as suggested.

7. Revised the confusing statement for Fig. 2d-e “NEK2 protein are mainly localized at the nucleus and cytosol”.
Revised.
8. The statement regarding to drug-resistance role of NEK2 confusing. The discussion is removed.

**Minor Revisions:**

1. **The writing needs to be improved.**
   The manuscript has been thoroughly revised by English-speaking person.

2. **The legend for Fig 2c should read ‘Scr’ not ‘Src’.**
   Corrected.

3. **The label of Fig3c is confusing.**
   Revised.

4. **A table is required for Fig 4a-b.**
   A table is added as suggested.

5. **Whether Fig 4a is a log-rank test should be descripted.**
   Revised as suggested.

6. **There are several statements in the discussion that require a reference.**
   The references are now added. )